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Electrical stimulation of left anterior thalamic nucleus with high-frequency and low-intensity currents reduces the rate of pilocarpine-induced epilepsy in rats

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ABSTRACT

Purpose: Bilateral electrical stimulation of anterior nuclei of thalamus (ANT) has shown promising effects on epileptic seizures. However, bilateral implantation increases the risk of surgical complications and side effects. This study was undertaken to access the effectiveness of a stimulation paradigm involving high frequency and low intensity currents to stimulate the left ANT in rats.
Methods: Male Sprague-Dawley rats were implanted with electroencephalogram (EEG) electrodes, and an additional concentric bipolar stimulation electrode into either the left or right ANT. The stimulus was a train of pulses (90 μs duration each) delivered with a frequency of 200 Hz and a current intensity of 50 μA. Thalamic stimuli were started 1 h before the first intraperitoneal pilocarpine injection (i.p., 300 mg/kg), and were applied for 5 h.
Results: EEG documented seizure activity and status epilepticus (SE) developed in 87.5% of rats treated with no ANT stimulation after a single dose of pilocarpine. Left ANT stimulation significantly increased the tolerance threshold for pilocarpine-induced EEG seizure activity; 20% of rats developed their EEG documented seizure activity after receiving the first dose, whereas 50%, 10% and 20% of rats did not develop seizure activity until they had received the 2nd, 3rd and 4th pilocarpine injection at 1-h intervals. Moreover, left thalamic stimulation reduced the occurrences of both EEG documented seizure activity and SE induced by single-dose pilocarpine to 25%. However, our result demonstrated that little effect on the occurrence rate of seizures and SE was found when rats received right ANT stimulation.
Conclusions: These results suggest that continuously 5-h left ANT stimulation with high frequency and low intensity currents, beginning from 1 h before the pilocarpine administration, may successfully reduce the occurrence rate of EEG documented seizure activity and SE development in rats.

1. Introduction

Epilepsy is one of the most common and devastating neurological disorders. About seventy percent of patients with epilepsy can be well-controlled with currently available anti-epileptic drugs (AEDs), but seizures still persist in 30% of epilepsy patients who do not respond to any of two to three first-line AEDs despite administration of the carefully optimized drug treatment.[1]

Many patients with epilepsy are inadequately controlled by the AEDs and also are not eligible for resective surgery. Alternative therapies, such as vagus nerve stimulation[2,3] and deep brain stimulation (DBS), have been considered for treating refractory epilepsy.

DBS has been used to treat various psychiatric (e.g., depression and obsessive-compulsive disorder)[4–7] and neurological disorders, such as Parkinson's disease (PD)[8] and epilepsy.[9–12] Stimulation of the subthalamic nucleus (STN) improves the cardinal features of PD, and the pedunculopontine (PPN) nucleus has recently emerged as a possible target of DBS for gait disorders in PD.[8] The targeted structures of DBS used for depression include the subthalamic nucleus, internal globus pallidus, ventral internal capsule/ventral striatum, the subgenual cingulated region, and the nucleus accumbens.[6] Amygdala and nucleus accumbens have been

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indicated as the targets of DBS for post-traumatic stress disorder (PTSD)[5] and refractory obsessive-compulsive disorder,[4] respectively. Bilateral electrical stimulation of the anterior nuclei of the thalamus (ANT) is promising in reducing epilepsy in animal experiments[11,12] and human studies.[9,10] The efficiency of the ANT stimulation in treating refractory epilepsy depends on the stimulation paradigm. Low frequency stimulation synchronizes electroencephalic activity in cortex[13,14] and is proconvulsant;[15] whereas high frequency stimulation desynchronizes intrinsic cortical activity[16] and raises seizure threshold.[15] Furthermore, unilateral DBS of the ANT has not been shown to reduce the propensity or latency for developing seizures and status epilepticus (SE).[11] However, bilateral implantation of electrodes into the ANT increases the complexity of surgery, the risk of surgery complication and adverse effects (e.g., intracranial and intracerebral hemorrhage, infection, misplacement of the DBS leads, or suboptimal placement of the leads).[17] We herein reported a stimulation paradigm by employing the relatively high-frequency (200 Hz) and low-intensity currents (50 μA) to unilaterally stimulate the left ANT, which successfully reduced the occurrence rate of pilocarpine-induced seizures and SE in rats.

2. Methods

2.1. Substances

Stock solutions of pilocarpine and methylscopolamine bromide (Sigma–Aldrich, St. Louis, MO, USA) were dissolved in pyrogen-free solution (PFS). These stock solutions were stored at −20 °C until administration. The dose of pilocarpine used in these experiments was 300 mg/kg with intraperitoneal (i.p.) injection. Methylscopolamine bromide (1 mg/kg, ip), an anti-cholinergic that does not cross the blood–brain barrier, was administered to reduce the peripheral cholinergic effects without affecting the central nervous system (CNS). Our personal observation demonstrated that rats would not survive after SE if the injection dose of pilocarpine is over 300 mg/kg. Therefore, we selected the dose of 300 mg/kg pilocarpine to provoke seizures.

2.2. Animals

Male Sprague-Dawley rats (250–300 g; National Laboratory Animal Breeding and Research Center, Taiwan) were used in these experiments. These animals were anesthetized (Zoletil® (Carros, France); 50 mg/kg), and injected with analgesic (morphine) and antibiotic (penicillin G benzathine). All rats were surgically implanted with three electroencephalogram (EEG) screw electrodes (on the right frontal and parietal lobes and the left occipital lobe) as previously described.[18] An additional concentric bipolar electrode (O.D. 0.125 mm, FHC, Bowdoinham, ME, USA) was implanted directly into the left ANT (AP, −2.0 mm from bregma; ML, 1.5 mm; DV, 5.5 mm)[19] in rats of groups 2–5 (see later in the experimental protocol). Insulated leads from EEG electrodes were routed to a Teflon pedestal (Plastics One, Roanoke, VA, USA). The Teflon pedestal was then cemented to the skull with dental acrylic (Tempron, GC Co., Tokyo, Japan). The incision was treated topically with polysporin (polymyxin B sulfate–bacitracin zinc) and the animals were allowed to recover for seven days prior to the initiation of experiments. The rats were housed separately in individual recording cages in an isolated room, in which the temperature was maintained at 23 ± 1 °C and the light:dark rhythm was controlled in a 12:12 h cycle (40 W × 4 tubes illumination). Food and water were available ad libitum. On the second postsurgical day, rats were connected to the recording apparatus (see later) via a flexible tether. Animals were habituated by daily handling timed to coincide with scheduled experimental administrations. We made our best effort to minimize animal suffering and to reduce the number of animals used in current study. All procedures performed in this study were approved by the National Taiwan University Animal Care and Use Committee.

2.3. Recording apparatus and ANT stimulation

Signals from the EEG electrodes were fed into an amplifier (Colbourn Instruments, Lehigh Valley, PA; model V75-01). The EEG was amplified (factor of 5000) and analog bandpass was filtered between 0.1 and 40 Hz (frequency response: ±3 dB; filter frequency roll off: 12 dB/octave). These conditioned EEG signals were subjected to analog-to-digital conversion with 16-bit precision at a sampling rate of 128 Hz (NI PCI-6033E; National Instruments, Austin, TX). The digitized EEG waveforms were stored as binary computer files pending subsequent analyses. Postacquisition determination of the onset of the first EEG seizure occurrence and the latency to SE was done by the visual scoring using AxoScope 10 Software (Molecular Devices, Sunnyvale, CA, USA). We defined EEG documented seizures as the visualization of epileptiform spikes with amplitudes of greater than 1 mV appearing in discharges lasting for at least 30 s. SE was defined as seizure activity associated with continuous epileptiform discharges followed by the periodic epileptiform discharges of at least 5 min duration.[20] EEGs were analyzed with the open-source Chronux algorithms (http://chronux.org/) run by the Matlab Signal Processing Toolkit for the fast Fourier transform (FFT) and multi-taper time-frequency spectrum.

Thalamic stimulation was started 1 h before pilocarpine i.p. injection and lasted for 5 h. A stimulator-isolator unit (A360 Stimulus Isolator, World Precision Instruments, Sarasota, FL, USA) triggered by a main stimulator (Accupulser A310, World Precision Instruments) was used to deliver the ANT stimulation current at a frequency of 200 Hz, pulse width 90 μs, interval 4.1 ms, and intensity 50 μA. The stimulation artifact was not observed during the stimulation time period because of the low sampling rate with which the EEG was acquired. However, we simultaneously determined the stimulation outputs by oscilloscope when the animals received ANT stimuli. The parameters of ATN stimulation with high-frequency and low-intensity currents would deliver the lowest possible total electrical energy delivered (TEED) than that of low-frequency and high-intensity currents.[21] The TEED is calculated as: $TEED_{1\,s}$ = (voltage^2 × frequency × pulse width/impedance) × 1 s.[21] The optimized DBS setting should generate maximal clinical benefit at the lowest possible TEED, which results in fewer stimulation-related complications. Furthermore, the ANT stimulation did not alter the quality of EEG signals as mentioned in the following result section.

2.4. Experimental procedures

A total of 50 Sprague-Dawley rats were used and divided into six groups. Control rats in group-1 ($n = 8$) received a single dose of pilocarpine administration at the second hour of the dark period. EEGs were recorded before and after pilocarpine injection. Rats in group-2 received a similar protocol as those in group-1, except that rats were implanted with a left ANT electrode but electrical stimulation was not delivered. In group-3 ($n = 10$), a 1-h baseline EEG was recorded at the beginning of the dark period. The continuous 5-h left ANT stimulation and EEG recording were simultaneously performed from the 2nd-hour of the dark period. The first i.p. administration of pilocarpine was given 1 h after the initiation of ANT stimulation. The second dose of pilocarpine was given 1 h later if the EEG epileptiform did not occur after the first pilocarpine injection. The third and fourth injections of pilocarpine were given at 1-h interval if the previous dose of pilocarpine did not cause EEG epilepsy. Rats in group-4 ($n = 8$) received the similar protocol as those in group-3, except that pilocarpine was administered once at

1-h after the beginning of the left ANT stimulation. Rats in group-5 ($n = 6$) had the similar protocol as those of group-4, except that rats received right ANT stimulation. Rats in group-4 and group-5 received the same DBS procedure, which was performed using the same equipment and by same investigators. The only difference was that the stimulation target in group-4 was the left ANT and the target in group-5 was the right ANT. Rats in group-6 ($n = 8$) received the similar protocol as those in group 3, except that pilocarpine was administered once at 30 min after the beginning of the left ANT stimulation. Rats from these six groups were sacrificed two days after the end of ANT stimuli, if animals survived; and the dissected brain tissue blocks were perfused with 4% paraformaldehyde for fixation. Then, 40 μm thick brain slices containing the ANT region were sliced coronally by cryotome at the same brain level and were used to verify the ANT lesion caused by stimulation electrode or electrical stimulation.

2.5. Statistical analyses

All values acquired from the EEG recordings were presented as the mean ± SEM for the indicated sample sizes. The nonparametric Mann–Whitney and Chi Square (χ^2) tests were used. An α level <0.05 was taken as indicating a statistically significant difference.

3. Results

3.1. Effects of pilocarpine-induced EEG epilepsy and behavior changes

Administration of pilocarpine induced severe behavioral signs of cholinergic effects, including piloerection, salivation, red eyes, shivering and facial automatisms, within 5 min in the group-1 rats. The severity of cholinergic behavioral signs gradually increased until the development of EEG seizures. The latency to develop the first EEG seizure was 12.3 ± 2.3 min obtained from seven out of eight rats (87.5%), which received a single-dose pilocarpine administration in group-1 (Fig. 1, Table 1). Furthermore, seven out of eight (87.5%) rats developed SE with a latency of 22.3 ± 5.0 min after the single-dose pilocarpine injection (Fig. 1, Table 1). This result indicated that 300 mg/kg pilocarpine is a dosage which reliably leads to the development of EEG documented seizures and SE in our model. None of the rats which developed SE survived.

3.2. Effects of left or right ANT stimulation given 1-h before pilocarpine administration

Left ANT stimulation did not alter EEGs when compared to the EEGs acquired from the undisturbed baseline, whenever rats were

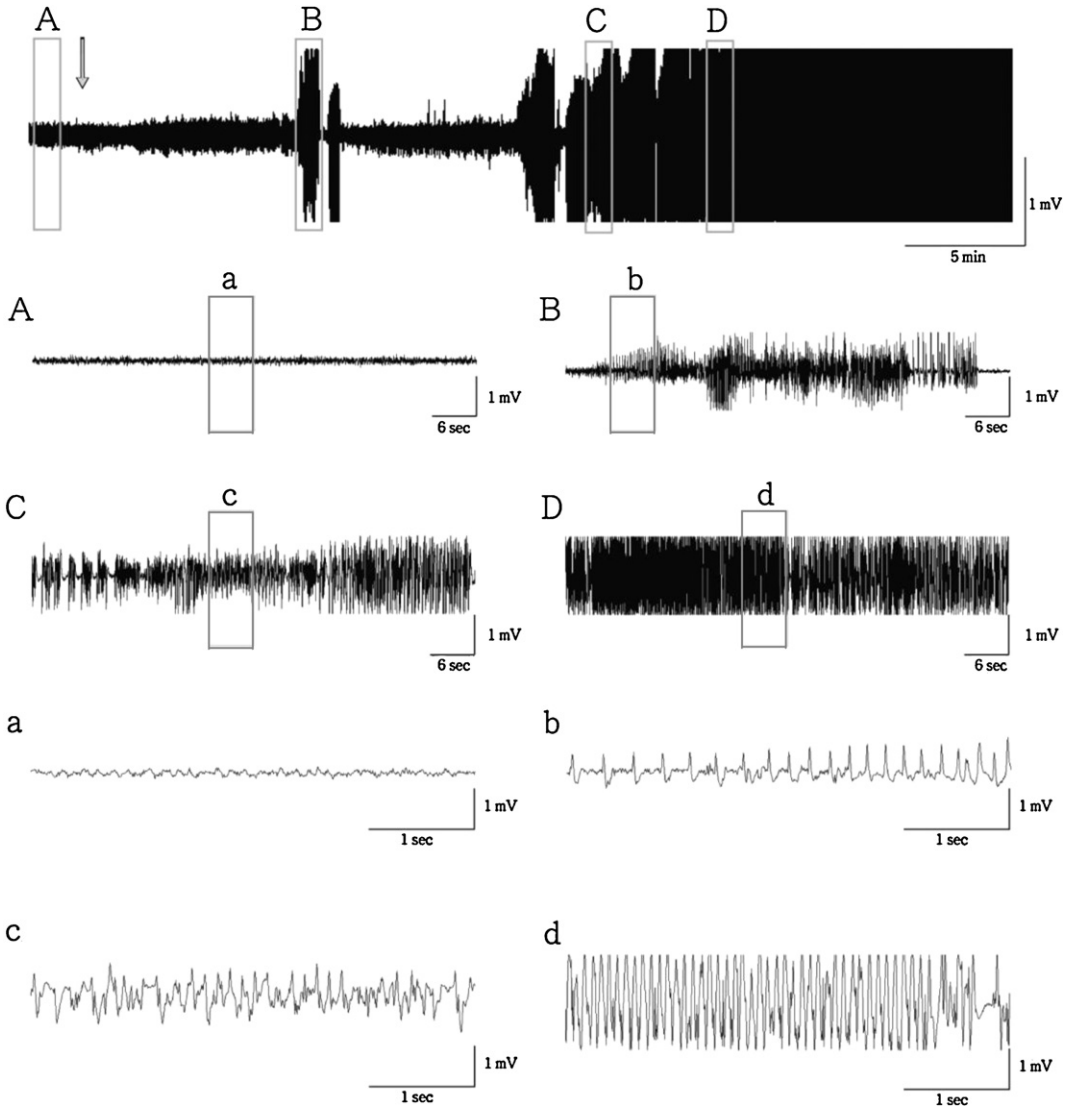

Fig. 1. EEG recordings before and after the pilocarpine administration. Arrow indicates that injection of pilocarpine. Panel A demonstrates the baseline EEGs obtained before the pilocarpine administration, panel B depicts the first EEG seizure, and panels C & D indicate the EEG with SE. Panels a–d are EEGs extracted from panels A–D.

Table 1
Effects of right or left ANT DBS on the occurrence rates of first EEG seizure and SE, the survival rate, and the latencies to develop first EEG seizure and SE.

Group	Total sample size	Number of rats develop EEG seizure	Number of rats develop SE	Number of rats survived	Latency to develop first EEG seizure	Latency to develop SE
Group 1 (pilocarpine control)	8	7	7	1	12.3 ± 2.3 min	22.3 ± 5.0 min
Group 2 (Sham control: Left ANT electrode implanted)	10	8	5	8	25.1 ± 7.9 min	41.3 ± 11.0 min
Group 3 (left ANT DBS with repeated pilocarpine injections)	10	2	2	8	Not determined[b]	Not determined[b]
Group 4 (left ANT DBS)[a]	8	2	2	8	23.4 ± 10.1 min	37.7 ± 14.4 min
Group 5 (right ANT DBS)	6	6	6	0	24.7 ± 7.7 min	47.8 ± 16.3 min
Group 6 (left ANT DBS)[a]	8	5	5	3	11.9 ± 1.8 min	17.4 ± 2.5 min

[a] Rats in group-4 received left ANT DBS 60 min prior to pilocarpine administration, whereas rats in group-5 received left ANT DBS 30 min before pilocarpine injection.
[b] Rats in group-3 received repeated pilocarpine administrations (see Section 2.4), therefore the latencies for developing first EEG seizure and SE were not determined.

in waking (Fig. 2A) or during slow wave sleep (SWS) in the group-4 (Fig. 2C). Further analyzing the EEG spectra during wakefulness and SWS indicated that no significant alteration was induced by the left ANT stimulation (Fig. 2B and D), suggesting that the present paradigm of thalamic stimulation did not alter spontaneous EEGs. The propensity for pilocarpine to induce EEG seizure and develop SE was reduced to 25% (two out of eight rats) when rats received left ANT stimulation 1 h before pilocarpine injection in the group-4 (Fig. 3, Table 1). The upper panel of Fig. 3 demonstrated an example of EEGs in which no epileptiform activity was observed when rats received the left ANT DBS. However, two out of eight rats did exhibit epileptiform EEGs despite treatment with the left ANT DBS as shown in the second panel. There was a statistically significant association between the left ANT DBS and the efficiency for reducing the rate of EEG seizure in rats ($\chi^2 = 12.30$, $p < 0.001$, when comparing to the control group). Furthermore, the association between the left ANT DBS and the efficiency for reducing the rate of SE in rats also demonstrated statistical significance ($\chi^2 = 12.30$, $p < 0.001$, when comparing to the control group). The onset of the first EEG seizure (23.4 ± 10.1 min) and the latency to develop SE (37.7 ± 14.4 min) were also prolonged in the two rats which received the left thalamic stimulation but still developed seizure and SE, although this observation failed to reach statistical significance when compared to the pilocarpine-treated control rats without receiving left ANT stimulation ($p = 0.182$ & 0.355, respectively; Mann–Whitney test). The reason for not achieving statistical significance between groups may be due to the small group sizes. Comparing to the effect of left ANT stimulation, all of 6 rats in the group-5, which received the right ANT DBS, developed pilocarpine-induced EEG seizures and SE (Table 1). However, the onset of the first

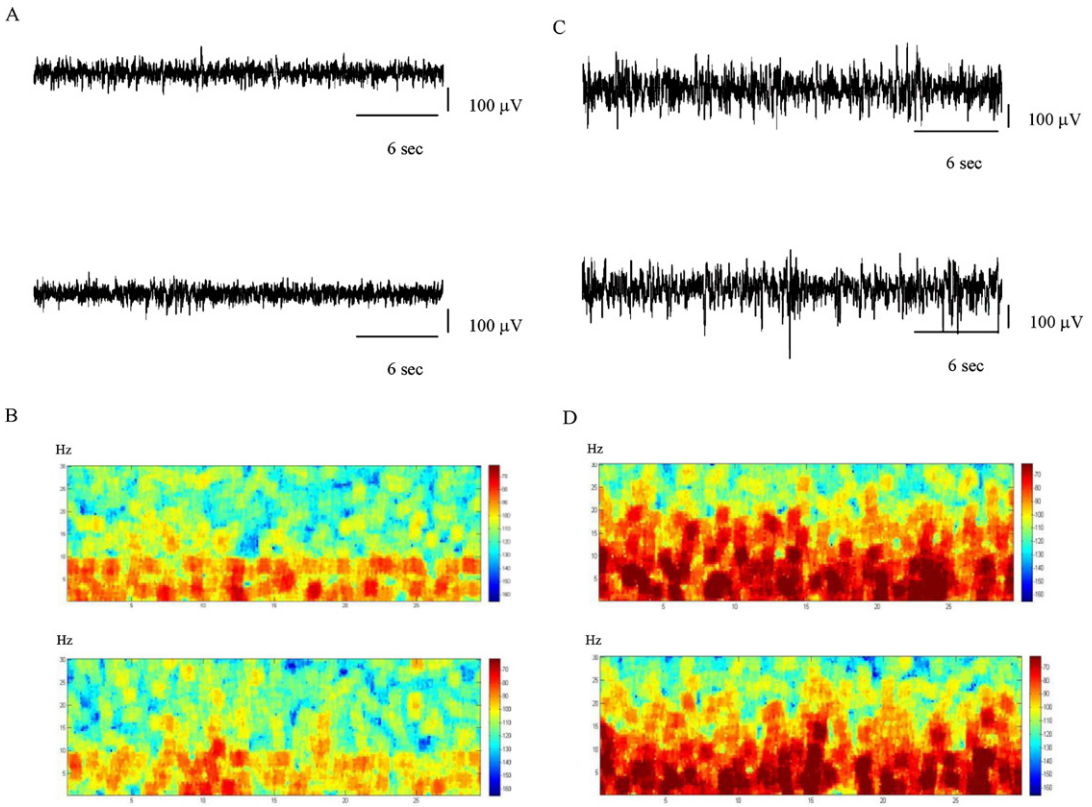

Fig. 2. Effects of left ANT DBS on baseline EEGs. A: Upper panel demonstrates the baseline waking EEG and lower panel is the EEG acquired during left ANT DBS. B: Upper panel depicts the spectral analysis from the baseline waking EEG; lower panel indicates the spectral analysis obtained from the waking EEG during left ANT DBS. Red: high intensity; blue: low intensity. C: Upper panel demonstrates the baseline SWS EEG and lower panel is the EEG acquired during left ANT DBS. D: Upper panel depicts the spectral analysis from baseline SWS EEG; lower panel indicates the spectral analysis obtained from the SWS EEG during left ANT DBS. Red: high intensity; blue: low intensity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

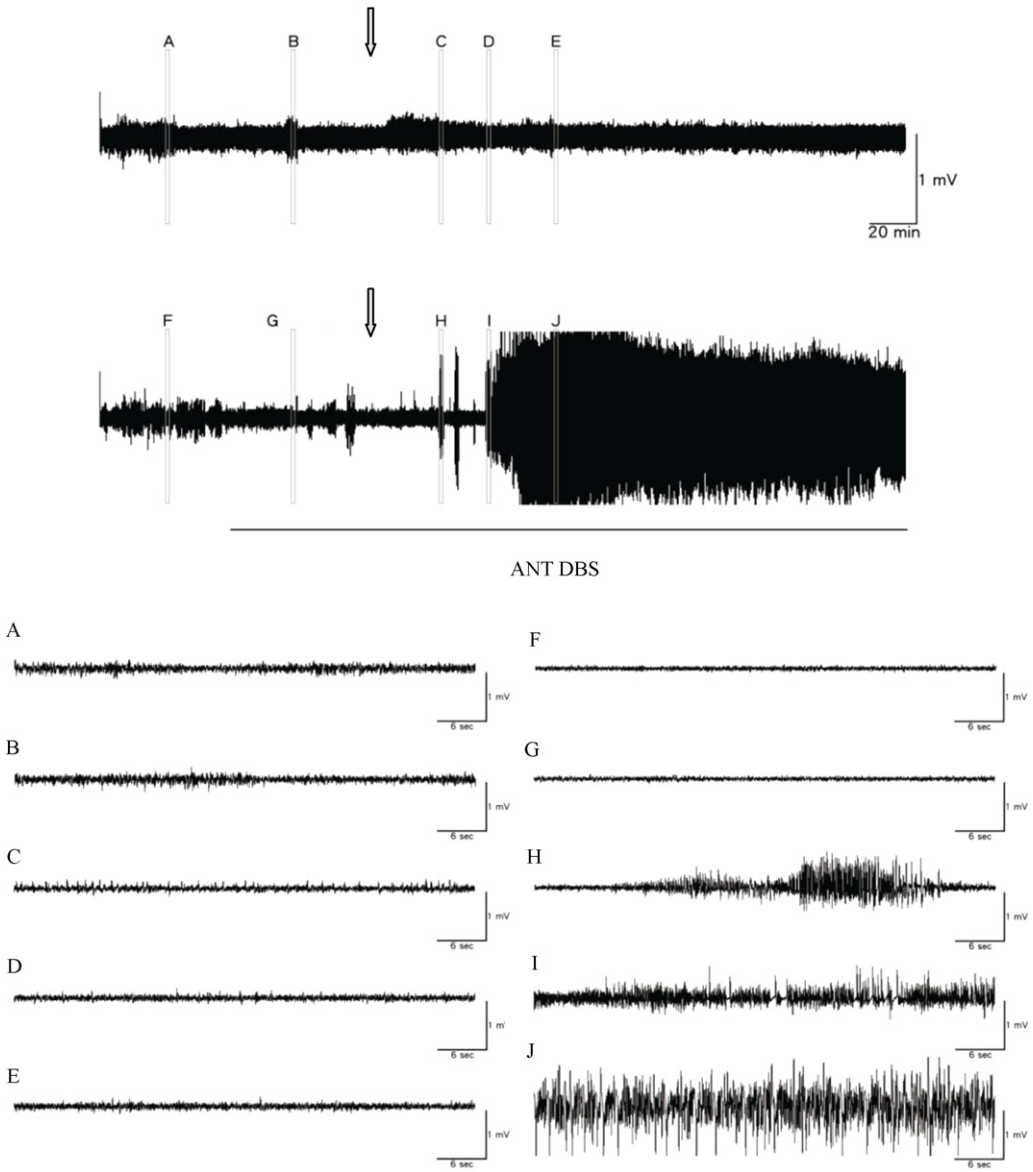

Fig. 3. Effects of left ANT DBS on pilocarpine-induced epileptogenesis. Upper panel is an example demonstrated that the left ANT DBS successfully suppressed pilocarpine-induced EEG seizure. Lower panel is a failure example for left ANT DBS to suppress epileptogenesis. Panels A–J are EEGs extracted from these two EEG traces. H: the EEG seizure, I & J: SE EEGs. Arrows indicate the administration of pilocarpine.

EEG seizure and the latency for developing SE were delayed to 24.7 ± 7.7 min ($p = 0.130$ when compared to the values obtained from group-1) and 47.8 ± 16.3 min ($p = 0.114$ when compared to the values acquired from group-1), respectively, after receiving right ANT stimuli, although these alterations did not reach statistical significance when compared to that of control group-1 (Table 1). Rats in group-2, which were implanted with a left ANT electrode but which did not receive any electrical stimulation, did not show a significant reduction of the occurrence rate of seizures. The latencies to first EEG seizure and SE were prolonged, but did not reach statistical significance (Table 1). Furthermore, left ANT stimulation significantly increased the tolerance threshold for pilocarpine to induce the occurrence of EEG seizures in the group-3 rats; 20% (2/10) of rats developed their first EEG seizure after receiving the first dose, whereas 50% (5/10), 10% (1/10) and 20% (2/10) of rats did not induce the first EEG seizure until receiving the 2nd, 3rd and 4th pilocarpine injection, respectively. Fig. 4 demonstrates that the first epileptiform EEG appeared after the third pilocarpine administration, and the SE occurred after the fourth injection.

3.3. Effects of left ANT stimulation given 30-min before pilocarpine administration

The propensity for one-dose of pilocarpine to induce EEG seizure and develop SE was 62.5% (five out of eight rats) when rats in the group-6 received left ANT stimulation 30 min before the pilocarpine injection. There was no statistically significant association between the left ANT DBS (starting 30-min before pilocarpine administration) and the efficiency for reducing the occurrence rate of EEG seizure and SE in rats. The onset of the first EEG seizure and the latency to develop SE were 11.9 ± 1.8 min ($p = 0.935$ when compared to the values obtained from group-1; Mann–Whitney test) and 17.4 ± 2.5 min ($p = 0.808$ when compared to the values obtained from group-1; Mann–Whitney test), respectively, which did not differ from those values obtained from rats received only pilocarpine administration.

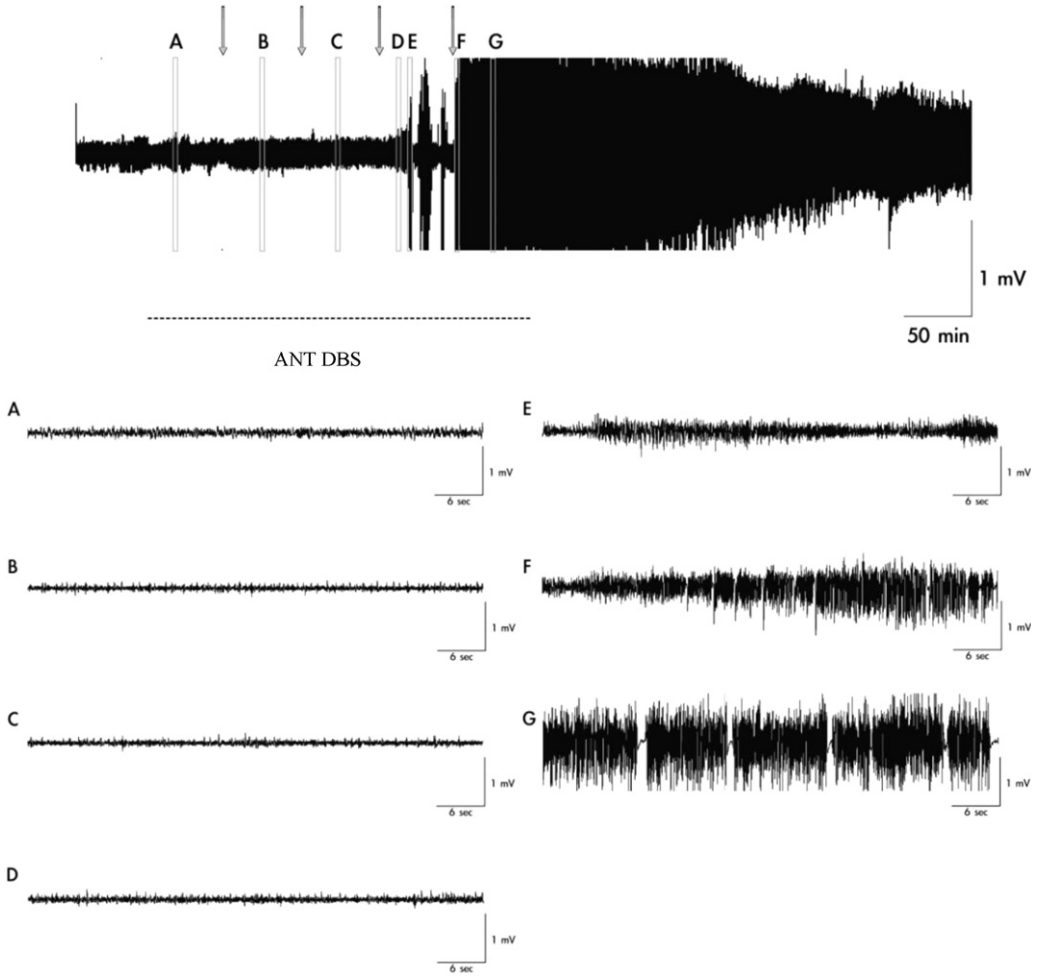

Fig. 4. Effects of left ANT DBS on the tolerance threshold for pilocarpine to induce the occurrence of EEG seizures. A: EEGs recorded before pilocarpine administration. B: EEGs acquired after the 1st pilocarpine injection. C: EEGs recorded after the 2nd administration. D & E: normal EEGs and epileptiform EEGs obtained after the 3rd injection. F & G: SE EEGs acquired after the 4th pilocarpine injection. Arrows indicate the administration of pilocarpine.

3.4. Histological examination of ANT after the ANT DBS

Our results demonstrated that the left ANT electrical stimulation did not cause obvious lesion in the ANT brain regions (Fig. 5). Fig. 5A indicated the ANT slice obtained from one representative rat in the control group-1, and Fig. 5B depicted the ANT region acquired from a rat with the 5-h ANT electrical stimulation (magnification: 100×) in the group-4. There was no lesion observed in a 400×-magnified slice obtained after the ANT DBS stimulation when compared with the slice acquired from the control rat (Fig. 5C and D). Nonetheless, the ANT stimulating electrode may have caused a minimal lesion, but it would not be realistic to claim that the electrode did not cause lesion at all. The microscopic lesion or the changes in the cellular and molecular levels by the electrode implantation or stimulation need to be further studied.

4. Discussion

4.1. Efficacy of DBS for epilepsy in humans and animal studies

Our results demonstrated that unilateral left ANT DBS with high-frequency and low-intensity currents successfully reduces the development of seizures and SE in an animal model. In the treatment of neurological diseases, DBS was initially used for movement disorders (e.g., Parkinson's disease)[22] and for chronic pain.[23] Recently DBS has become a promising therapy for patients with refractory epilepsy who do not respond adequately to AEDs or are not eligible for resective surgery. The first trial to assess the effect of DBS in epilepsy was carried out by Cooper et al. in 1970s, in which the seizure frequency was reduced by subdural stimulation of the cerebellum.[24–26] Several targets of deep brain structures, including the anterior thalamus,[27] the centromedian thalamic nucleus,[28] the caudate nucleus,[29] the posterior hypothalamus,[30] the hippocampus,[31] and the STN,[32] have been stimulated to try and suppress seizures. The ANT receives afferents from the mammillary bodies and subiculum via the mammillothalamic tract and the fornix, respectively. The efferent of ANT projects to the prefrontal cortex and cingulate gyrus,[33,34] which subsequently projects to the parahippocampal gyrus and entorhinal cortex.[33] The ANT also has direct projections to the retrohippocampal regions, which links the hippocampal formation with the neocortical association areas.[35,36] The mesial temporal lobe plays a primary role in the genesis of temporal lobe epilepsy (TLE). The mesial temporal lobe is composed of the hippocampus, the amygdala and the parahippocampal regions (e.g., entorhinal cortex, perihinal cortex and posterior parahippocampal cortex).[37] Therefore, the ANT is an important relay nucleus of the Papez circuit which exhibits an ability to manipulate (aggravate or suppress) the epileptogenesis of TLE.

The rationale for DBS suppressing epileptogenesis is based upon animal experiments and clinical studies in humans. In animal

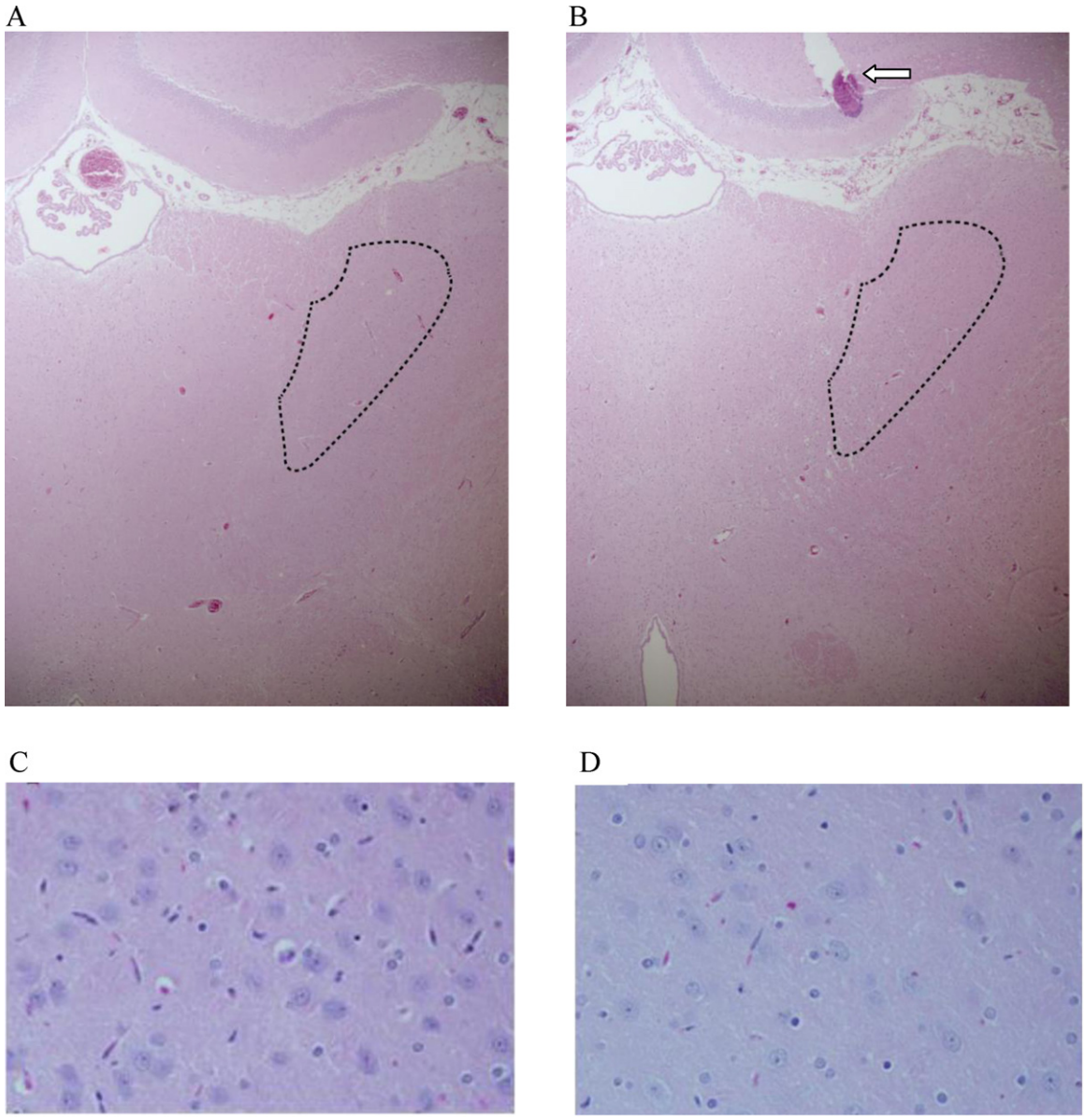

Fig. 5. Effects of electrode implantation and electrical stimulation on the ANT lesion. A: The slice (100×) of ANT obtained from one representative rat in control group-1. B: The slice (100×) of ANT obtained from a rat treated with the left ANT DBS in the group-4. Areas marked indicate the left ANT region. Arrow depicted the track of stimulation electrode. C: The slice (400×) of ANT obtained from one representative rat in control group-1. D: The slice (400×) of ANT obtained from a rat treated with the left ANT DBS in the group-4.

study, Dempsey and Morrison (1942) have shown that electrical stimulation of thalamus with lower frequency synchronizes rhythmically recurrent cortical potentials in several cortical regions.[13] In contrast, bipolar electrical stimulation of medial thalamic nuclei with high frequencies (200 Hz) desynchronizes intrinsic cortical activities in the motor and somesthetic areas in rabbits, which is similar to the result of reticular arousal activation.[16] Furthermore, the slow rhythms and the spindle activity simultaneously disappeared after receiving the high-frequency thalamic stimulation.[16] The effects of thalamic stimulation have been further confirmed in human studies. Electrical stimulation of centromedian thalamic nucleus with a current of low frequency (6 Hz) and high intensity (320–800 μA) elicits incremental, desynchronizing and spike-wave electrocortical responses with a bilateral regional scalp distribution.[38] Stimulation of centromedian thalamic nucleus with high frequency (60 Hz) and high intensity (320–800 μA) currents desynchronizes EEG and exhibits a slow negative shift of the EEG baseline.[38]

4.2. Complications/limitations of high-intensity bilateral DBS stimulation

Bilateral thalamic stimulation with a high-intensity current (320–800 μA) in animals[11] and in humans[38] is necessary for ensuring the efficacy of anti-epileptogenesis. Some reports even used a high-voltage (5 V) stimulation of ANT to achieve the successful anti-epileptogenetic effect.[10] However, the bilateral implantation of stimulation electrodes into the ANT may increase the risk of surgery complication and the chance of suffering from adverse effects (e.g., intracranial and intracerebral hemorrhage, infection, misplacement of the DBS leads, or suboptimal placement of the leads).[17] Furthermore, ANT DBS with high-intensity or high-voltage currents may impair the cognitive function and memory,

since the ANT is an important relay nucleus in the circuitry of Papez for memory. An animal study demonstrated that the ANT stimulation at relatively high current (500 μA) disrupts the acquisition of contextual fear conditioning and impairs performance on a spatial alternating task in rats.[39] Therefore, finding an effective and feasible stimulation paradigm of the unilateral ANT DBS stimulation is necessary for employing this technique in the treatment of refractory epilepsy. We herein hypothesized that application of a unilateral ANT DBS with high-frequency (200 Hz) and low-intensity (50 μA) currents at 1 h or 30 min before the pilocarpine administration could suppress pilocarpine-induced epileptogenesis.

4.3. Previous attempts to treat seizures with unilateral ANT stimulation

Several studies indicate that bilateral high-frequency stimulation of ANT and lesion of ANT are protective against the occurrence of seizure and SE.[11,15] Bilateral ANT stimulation at a frequency of 100 Hz, pulse width 100 μs, and intensity 800 μA, started 5 min prior to pilocarpine administration, prolongs the latency to develop SE; however, unilateral ANT stimulation exhibits no effect on the pilocarpine-developed SE.[11] Another study has also demonstrated that high frequency stimulation (150 Hz, 450–800 μA) of bilateral ANTs, performed immediately before or after amygdala kindling-induced seizure, reduces the incidence of seizure occurrence.[40] However, unilateral high-frequency stimulation of ANT fails to inhibit kindling-induced seizures.[40] These observations suggest the efficacy of bilateral ANT DBS, rather than that of unilateral ANT DBS, on the inhibition of seizure and SE. Nevertheless, two recent studies revealed the effect of unilateral ANT DBS on reducing seizure and SE in animal models. Takebayashi et al. (2007) have demonstrated that unilateral ANT stimulation at a frequency of 130 Hz, pulse width 100 μs, and intensity 140–500 μA, delivered after injection of kainic acid into the left sensorimotor cortex, suppresses focal cortical seizure.[41] Furthermore, unilateral ANT DBS at a frequency of 200 Hz, pulse width 100 μs, and intensity 450–800 μA, given before kindling stimulation and lasted for 15 days, decreases the incidence of generalized seizures and after discharge duration induced by amygdala kindling stimuli.[42]

Our current results indicate that high-frequency unilateral ANT DBS did not disturb the baseline EEGs acquired from states of wakefulness and SWS as shown in Fig. 2. There was no significant artifact observed in the waking and sleep EEGs during the unilateral ANT DBS. Furthermore, analysis of EEG spectra before and after unilateral ANT DBS depicted that no alteration was found, suggesting the high-frequency unilateral ANT DBS has limited adverse effect on normal baseline EEGs. Pilocarpine administration induced epileptiform EEG seizure in 87.5% of rats, and 87.5% of rats developed SE after administration. None of the rats which developed SE survived. This result indicated that 300 mg/kg pilocarpine is a feasible dosage to develop EEG seizure and SE in our current study. Both the occurrence rates of pilocarpine-induced epileptogenesis and SE were reduced to 25% when left ANT DBS was employed 1 h before the pilocarpine administration and lasted for 5 h. The onset of the first EEG seizure and the latency to develop SE were prolonged in the two out of eight rats which received the left thalamic stimulation but still developed seizure and SE. However, our result demonstrated that little effect on the occurrence rate of seizure and SE was found when rats received the right ANT stimulation. There is no biologically plausible explanation of why the right ANT DBS exhibits no effect of seizure suppression. Although the right ANT DBS was not effective in suppression of seizure occurrence, it prolonged the latency for the development of seizure and SE. With such small sample sizes, $n = 6$, a high degree of variability is to be expected and significant differences can occur by chance. It is unclear if a larger number of animals could have led to significant difference. Furthermore, the left ANT DBS became less efficient in suppressing the occurrence rate of EEG seizure and SE, if the stimulation was starting at 30 min prior to pilocarpine administration. This observation suggests that giving the current paradigm of left ANT DBS to reduce the occurrence rate of seizures with a certain period of time (e.g., 60 min) prior to the pilocarpine administration is required. Furthermore, the 5-h left ANT DBS starting at 1 h before the 1st pilocarpine injection increased the tolerance threshold for pilocarpine to induce the occurrence of EEG seizures. These results suggest that the pilocarpine-induced epileptogenesis can be successfully antagonized by unilateral left ANT DBS with high-frequency (200 Hz) and low-intensity (50 μA) currents. This efficacy of unilateral left ANT DBS on seizure suppression is contradictory to the other studies.[11,40] We proposed the difference might be due to the timing for administering unilateral ANT DBS. In the previous studies with no efficacy of unilateral ANT DBS on seizure suppression, the timing for administering unilateral ANT DBS is either 5 min prior to the pilocarpine injection[11] or immediately before/after amygdala kindling stimuli.[40] Our current results elucidated that left thalamic stimuli delivering 30 min prior to the pilocarpine administration lost its ability to reduce seizures and SE, suggesting that the left ANT DBS should be employed at least 30 min prior to the seizure onset to ensure its efficacy. One study demonstrates that unilateral ANT DBS delivered 15 days before amygdala kindling stimulation successfully decreases the incidence of seizure and after discharge duration,[42] which is consistent with our current findings. Furthermore, the optimized DBS setting should generate maximal clinical benefit at the lowest possible TEED, which results in fewer stimulation-related complications. The stimulation intensity and TEED we used in this study to reduce the rate of seizure and SE is much lower than others,[41,42] suggesting that our parameters of left ANT DBS for seizure reduction is feasible and could minimize the stimulation-related complications.

4.4. Limitations

Although this DBS paradigm did not affect the baseline EEG activities and spectra during stimulation, the alteration of synaptic plasticity after the long-term and high-frequency DBS may occur and the effect of changing in the synaptic strength needs to be considered in future study. In addition to the possible effect on synaptic strength, the ANT lesion caused by DBS may also influence the outcome of ANT DBS on anti-epileptogenesis. One study reported that neither pilocarpine-induced generalized seizures nor SE was developed after the bilateral thalamotomy, but both seizures and SE was still developed by pilocarpine in rats received the unilateral lesion.[11] However, we did not observe significant lesion after the ANT DBS in our study. Nevertheless, micro-thalamotomy or lesion may occur, and it needs to be further confirmed. One might also concern the effect of implantation of ANT stimulating electrode with no electrical stimulation on the pilocarpine-induced seizures. Our result (Table 1) indicated there is no significant difference in the occurrence rate of EEG seizures between rats received pilocarpine injections (group-1) and those received pilocarpine administration with implantation of an ANT stimulating electrode (group-2).

4.5. Potential translation into humans

Recent DBS would only be applied after epilepsy patients exhibit the initial epileptogenic insult. According to our current results, unilateral left ANT DBS has to be employed 1 h before the

seizure onset to reduce the incidence of seizure and SE, which makes the prediction of seizure onset more critical in the DBS therapy. Our collaborators are currently designing a mathematical algorithm to analyze EEGs obtained before epilepsy onset and to predict the onset of epilepsy. Our results indicate that the mathematical algorithm we created could successfully predict the occurrence of EEG seizure 1 h before the seizure onset (personal unpublished data). Combining our predicting algorithm with high-frequency and low-intensity left ANT DBS may efficiently improve patients with refractory epilepsy in clinic.

Disclosure of conflicts of interest

Authors have indicated no financial conflicts of interest.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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